

CLAIMS

1. A presentation system for use in quantifying an amount of a target moiety which is present in a sample, the presentation system comprising at least one copy of a target moiety or part thereof that is recognisable by a binding partner and at least one domain of a scaffold covalently linked to said target moiety, said domain being non-reactive to a binding partner specific to said target moiety or part thereof.
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2. A presentation system according to claim 1 wherein the copy of the target moiety or part thereof is selected from the group comprising sequences of DNA or RNA, a peptide, an antigenic structure or a chemical entity or moiety.
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3. A presentation system according to either preceding claim wherein the target moiety further includes saccharides, metabolite cofactors, haptens or a modification by a phosphate, nitrosylated group, sulphated group or glycosylphosphatidyl inositol (GPI) group.
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4. A presentation system according to any preceding claim wherein the scaffold material of the presentation system has a controllable property.
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5. A presentation system according to claim 4 wherein the controllable property is relative molecular mass (Mr) or weight (Mwt) or isoelectric point (pI).
6. A presentation system according to any preceding claim wherein the scaffold material is a protein.
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7. A presentation system according to any preceding claim wherein the scaffold material comprises at least one natural or unnatural amino acid with at least one or more chemically groups within a side chain of a residue.

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8. A presentation system according to claim 7 wherein the chemically reactive acidic group is selected from the group comprising a carbonyl on glutamic acid, aspartic acid, a hydroxyl on tyrosine, cysteine and lysine.
- 5 9. A presentation system according to any preceding claim wherein the scaffold comprises one or more chemically reactive cysteine and/or lysine amino acid residues.
- 10 10. A presentation system according to claim 9 wherein the number of reactive cysteine and/or lysine residues is controlled by either selecting the scaffold protein from a natural source which contains a desired number of reactive cysteine and/or lysine groups or by selectively mutating in or out a reactive residue from a protein sequence or rendering ineffective any one or more of the reactive cysteine and/or lysine residues of a selected scaffold protein.
- 15 11. A presentation system according to any preceding claim wherein the scaffold is selected from the group comprising: I27 domain from titin; I39 domain which is a subunit (subunit 5) of splicing factor 3b; organ of Corti protein (*Mus musculus*); heat shock protein, mitochondrial (*Mus musculus*); splicing factor 3B subunit 5 (*Mus musculus*); ubiquinol-cytochrome C reductase complex ubiquinone-binding protein; E1B protein (Human adenovirus type 11); chaperonin (*Arabidopsis thaliana*); photosystem II reaction center H protein (*Arabidopsis thaliana*); a NADH-ubiquinone oxidoreductase subunit, mitochondrial [Precursor] (*Homo sapiens*); signal recognition particle protein (*Mus musculus*); and DNA polymerase delta subunit 4(*Mus musculus*).
- 20 12. A presentation system according to claim 11 comprising a combination of any one or more of the scaffolds of claim 11.
- 25 30 13. A presentation system according to claim 11 comprising a plurality of identical scaffolds.

14. A presentation system according to claim 11 claim comprising a plurality of identical or non-identical domains as a mixture or blend thereof.
15. A presentation system according to claim 11 comprising a plurality of scaffold I27 domains from titin and wherein where at least one domain or unit is engineered to possess a single cysteine residue for peptide attachment while all other or a selected number of I27 domain or unit lacks cysteine residue(s) or other reactive residues selected from the group comprising lysine, glutamate and aspartate.
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- 10 16. A presentation system according to claim 11 comprising at least one or more scaffold I39 domain(s) which is/are a subunit (subunit 5) of splicing factor 3b.
- 15 17. A presentation system according to any preceding claim wherein the scaffold domains are about 10kDa.
19. A presentation system according to any preceding claim wherein the scaffold linear units or domains are linked in tandem.
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20. A presentation system according to any preceding claim comprising at least one biological or non-biological polymer.
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21. A presentation system according to claim 20 wherein the non-biological polymer is PEG (polyethylene glycols).
22. A presentation system according to any preceding claim wherein the copy of the target moiety is incorporated into the presentation system using either covalent attachment through a thiol group on a cysteine residue or in the instance of the target moiety being a protein or peptide and the presentation system is also a protein or peptide, a DNA segment encoding the target moiety is incorporated into the DNA
30 encoding the presentation system, and subsequently expressed with the presentation system.

23. A presentation system according to any preceding claim wherein the domains of the presentation system, apart from the copy of the target moiety or part thereof, are substantially inert or are non-reactive to the specific binding partner of the sample target moiety or part thereof.

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24. A presentation system according to any preceding claim wherein the copy of the target moiety or part thereof is not identical to the target moiety present in the sample.

10 25. A presentation system according to any preceding claim comprising a plurality of copies of the target moieties.

26. A presentation system according to any preceding claim wherein the copy of the target moiety or part thereof is linear or branched within the presentation system.

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27. A presentation system according to claim 26 wherein the copy of the target moiety is branched so that the covalent attachment is via a side chain of the scaffold material.

20 28. A presentation system according to any preceding claim wherein the binding partner is selected from the group comprising monoclonal antibodies, polyclonal antibodies, RNA or DNA or peptide aptamers or other antibody equivalents, dyes, drugs and metal chelates.

25 29. A presentation system according to any preceding claim comprising at least one I27 domain and/or at least one I39 domain and a target moiety selected from the group comprising A1, PS-38 or PT17 peptide.

30. Use of a product in quantifying the amount of a target moiety which may be present in a sample, the product comprising a plurality of presentation systems, each presentation system comprising at least one copy of a target moiety or part thereof

and at least one domain covalently linked to said copy of the target moiety, wherein the domain(s) is/ are non-reactive to a binding partner specific to said copy of the target moiety or part thereof, further wherein each presentation system has a different molecular weight from other presentation systems in the product.

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31. Use according to claim 30 as a positive control or internal standard or in generating a calibration curve.

10 32. A kit for quantifying the amount of a target moiety in a sample, the kit comprising a presentation system according to any of claims 1 to 29.

33. A method of quantifying the amount of target moiety in a sample which may contain the target moiety, the method comprising:

15 a) providing a presentation system which comprises at least one copy of the target moiety or part thereof that is recognisable by a binding partner and at least one domain which is non-reactive to said binding partner, said at least one copy of the target moiety being covalently bonded to the at least one domain of a scaffold;

20 b) carrying out a separation detection technique on said presentation system, wherein said presentation system is present in a specific amount;

c) generating at least one comparison point comprising intensity of a signal produced by the presentation system versus the amount of the presentation system.

25 34. A method according to claim 33 wherein the presentation system is present in a single specific amount.

35. A method according to claim 33 wherein the presentation system is present in a series of varying amounts.

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36. A method according to claim 35 wherein the varying amounts are in the same or different lanes or channels of a blot.

37. A method according to either of claims 35 or 36, wherein the comparison 5 point is a plurality of comparison points which together provide a calibration curve.

38. A method according to any of claims 35 to 37 claim further comprising comparing the comparison point or comparison points with the sample to quantify the amount of target moiety present in the sample.

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39. A method according to any of claims 33 to 38, wherein said presentation system is of a known molecular weight or pI.

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40. A method according to any of claims 33 to 39, wherein the presentation system comprises a non-biological polymer, a nucleic acid molecule, a peptide, protein or combinations thereof.

41. A method according to any of claims 33 to 40, wherein the presentation system comprises a plurality of domains linked in tandem.

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42. A method according to any of claims 33 to 41, wherein the presentation system comprises identical units or domains or non-identical or different units or domains.

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43. A method according to any of claims 33 to 42 wherein the unit(s) of the presentation system are non-reactive to the binding partner specific to the target moiety of part thereof.

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44. A method according to any of claims 33 to 43 wherein the copy of the target moiety or part thereof comprises sequences of DNA, RNA, protein or peptide,

saccharides, haptens, phosphate, nitrosylated groups, sulphated groups, GPI groups, an epitope, an antigenic structure or a chemical entity.

45. A method according to claim 44 wherein the copy of the target moiety
5 comprises SERCA2a or SERCA2a phosphorylated on serine-38.

46. A method according to any of claims 33 to 45 wherein the presentation system comprises differing target moieties or parts thereof.

10 47. A method according to any of claims 33 to 46, wherein the copy of the target moiety or part thereof is linear or branched within the presentation system.

15 48. A method according to any of claims 33 to 47, wherein the specific binding partner comprises a molecule which has a specific binding affinity for the target moiety and is capable of binding thereto.

49. A method according to claim 48 wherein the binding partner comprises an antibody, DNA sequence, RNA sequence, a dye, a metal chelate or a drug molecule.

20 50. A method according to any of claims 33 to 49, wherein the separation based detection technique comprises a dot blot, Western blot, RIA, fluorescence polarisation, ELISA, Northern blotting, Southern blotting, PCR, High Performance Liquid Chromatography (HPLC), capillary electrophoresis, 1D electrophoresis, isoelectric focusing, mass spectrometry or combinations of the above.

25 51. A method according to any of claims 33 to 50 wherein the presentation system acts as a positive control for detecting the presence or absence of a target moiety in a sample.

30 52. A method according to any of claims 33 to 50 wherein the presentation system acts as an internal standard by providing a one point calibration.

53. A method according to any of claims 33 to 50 wherein the presentation system is used to generate multiple comparison points so as to provide a calibration curve.

5 54. A method according to any of claims 33 to 50 wherein the presentation system is used to monitor efficiency of immunoprecipitation and/or stages of an immunoprecipitaion process.

10 55. A method according to any of claims 33 to 54 further including any one or more of the features of claims 2 to 29.

56. A method for quantifying an amount of a protein epitope in a sample, said method comprising:

15 (b) providing a protein presentation system comprising at least one copy of the protein epitope and at least one further protein domain, wherein said presentation system is of known molecular weight;

b) carrying out a Western blot experiment on said presentation system, wherein said presentation system is in a specific concentration; wherein said Western blot experiment utilises a binding partner specific to the target moiety; and further wherein said protein domain of the presentation system is non-reactive to the binding partner; and

20 c) generating a comparison point comprising intensity of a signal produced by the presentation system in said technique versus the concentration of the presentation system.

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57. A method according to any of claim 56 further including any one or more of the features of claims 2 to 29.

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